

Tissue Destruction Induced by *Porphyromonas gingivalis* Infection in a Mouse Chamber Model Is Associated with Host Tumor Necrosis Factor Generation

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Intrachamber challenge with *Porphyromonas gingivalis* strain 381 in a mouse subcutaneous chamber model results in a local infection that progresses to exfoliation of the chambers within 15 days. This study was designed to elucidate the contribution of host reactions to tissue destruction manifested by chamber exfoliation in animals infected with *P. gingivalis*. Chamber fluids showed increasing levels of prostaglandin E₂ with infection, and the levels of tumor necrosis factor (TNF) in chamber fluids peaked just before chamber exfoliation. Intraperitoneal injection of a TNF inhibitor, thalidomide (TH), reduced the number of exfoliated chambers, while indomethacin had no effect. Exogenous TNF in chambers without bacterial infection did not cause chamber exfoliation but induced neutrophil infiltration. In a dual-chamber model, two chambers were implanted in the same mouse. One chamber was infected with *P. gingivalis*, and 9 days later exogenous TNF was added to the other chamber. Altogether, 66.67% of uninfected chambers were exfoliated between day 11 and day 16, although no bacteria were recovered from uninfected chambers. TH treatment alleviated both infected and uninfected chamber exfoliation. In this study, tissue destruction caused by *P. gingivalis* 381 infection was due to the elevation of the TNF levels and not due to local bacterial activities. Our results further indicate that local infection by *P. gingivalis* 381, a nondisseminating strain, actually has systemic effects on the host pathological outcome.

It is clear that bacteria are essential for the initiation of periodontitis (11). Periodontal pathogens produce a variety of enzymes and toxins that can damage the tissues and initiate inflammation (8, 19, 28). Among putative periodontitis pathogens, strong evidence supporting the hypothesis that the virulence factors of *Porphyromonas gingivalis* are responsible for tissue destruction has accumulated (9), and data indicate that enzymes and cell wall components from *P. gingivalis* are able to destroy the extracellular matrix of the gingiva and to activate osteoclastic resorption of bone (26). This organism also produces noxious waste products that further irritate the tissue (3).

Once the immune and inflammatory processes are initiated, various inflammatory molecules, such as cytokines, prostaglandins, and host enzymes, are released from inflammatory cells and other host cells in the tissues (4, 14). These soluble factors further increase the extent of inflammation and exaggerate tissue destruction. It is well known that proinflammatory cytokines produced by host cells play an important role in periodontal tissue destruction (6). The cytokines derived from the various host cells in response to *P. gingivalis* infection are believed to act not only in host defense but also in periodontal tissue breakdown in plaque-associated periodontitis (10, 18).

Therefore, both bacterial products and host inflammatory mediators contribute to the clinical outcome of tissue destruc-

tion by periodontitis as measured by loss of periodontal attachment (2). Extensive data indicate that most damage to the extracellular matrix and bone destruction in periodontitis are the result of direct action of host-derived enzymes, cytokines, and other mediators (17).

A major challenge for researchers is how to evaluate the contributions of the bacterial capacities and host inflammatory reactions to the tissue destruction. In this study we developed a modification of a mouse chamber model (5), the dual-chamber model (DCM), with which we began to distinguish the contributions of bacterial and host factors. The goal of this study was to investigate the role that the host response plays in the capacity of bacterial infection to stimulate tissue destruction. Using the subcutaneous chamber model, we characterized the inflammatory mediator profile at the sites of infection by a periodontal pathogen, *P. gingivalis*. Evidence presented here established that there is an association between elevated titers of tumor necrosis factor (TNF) and the occurrence or progression of host tissue destruction, even at infection-free sites. Our results suggest that much of the damage seemingly caused by *P. gingivalis* infection actually is the result of stimulation of the host response rather than a direct effect of bacterial products. The data presented here further support the idea that periodontitis pathogens initiate the disease by activating host mechanisms that then destroy the supporting structures of the periodontium.

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MATERIALS AND METHODS

Bacterial strain and growth conditions. *P. gingivalis* strain 381 was cultivated on brucella blood agar plates using defibrinated sheep blood and hemolyzed

sheep blood cells with hemin and menadione in a 5% CO₂-10% H₂-85% N₂ atmosphere at 37°C. Bacteria were passaged by picking colonies at least five times on blood agar plates before inoculation into mouse chambers. The bacterial cells were harvested from 24- to 30-h cultures and resuspended in brain heart infusion (BHI) broth to an optical density at 600 nm of 1.0 by using a spectrophotometer, which corresponded to 1×10^9 CFU per ml. The bacterial cells were further concentrated by centrifugation at $1,000 \times g$, and the total volume was reduced to 1/10 the original volume.

Experimental animals and infection protocol. In BALB/c female mice that were approximately 8 weeks old coils were surgically implanted in the dorsolumbar region to create subcutaneous chambers (5). The coils were made with stainless steel surgical wire that was 10 mm long and 5 mm in diameter. After healing for at least 14 days, the animals were infection challenged by transcutaneous, intrachamber injection on day 0 of overnight-grown *P. gingivalis* 381 (1×10^9 CFU in 100 μ l BHI, unless indicated otherwise) or sham challenged (100 μ l BHI) as a control. Thalidomide (TH) purchased from Celgene (Summit, NJ) was injected intraperitoneally (100 μ g per mouse) in 100 μ l normal phosphate-buffered saline (PBS) daily following *P. gingivalis* infectious challenge until chamber exfoliation occurred or until at least day 25 after the bacterial challenge. The same protocol was applied to indomethacin (IN) obtained from Sigma Chemical (St. Louis, MO), except that the dose was 500 μ g for each mouse. All experiments were performed in accordance with protocols approved by the Animal Care and Use Committee of Chung Shan Medical University, Taiwan.

DCM experiments. Mouse TNF was purchased from Endogen (Rockford, IL), and prostaglandin E₂ (PGE₂) was purchased from Sigma. Both *Escherichia coli* O55:B5 lipopolysaccharide (LPS) and *N*-formyl-Met-Leu-Phe (fMLP) were purchased from Sigma. For the DCM, two chambers were implanted into each animal simultaneously on the back, and the chambers were at least 10 mm apart. *P. gingivalis* (1×10^9 CFU in 100 μ l BHI) was injected into infected chambers on day 0. Uninfected chambers were treated daily after day 9 with TNF (10 ng/chamber), LPS (25 μ g/chamber), fMLP (500 ng/chamber), live *P. gingivalis* (1×10^9 CFU/chamber), or heat-killed *P. gingivalis* (1×10^9 CFU/chamber). Overnight-grown *P. gingivalis* 381 was collected from anaerobic brucella blood agar plates. After centrifugation and resuspension in BHI, all the bacteria in test tubes were killed by using boiling water for at least 30 min. The death of bacteria was confirmed by recultivation in anaerobic brucella blood agar plates.

Chamber fluid analysis. To minimize the impact of repeated sampling from chambers on the experimental outcome, chamber fluids were aspirated with 10 μ l daily using 26-gauge Hamilton microtiter syringes (Hamilton, NE) and diluted 1/50 in PBS. The chamber fluids were centrifuged at $300 \times g$ for 10 min. The pellets were removed, and the supernatants were stored at -80°C until they were analyzed.

Quantification of bacteria in chamber fluids. Serial dilutions of fluid samples were streaked on anaerobic blood agar plates and cultivated in a 5% CO₂-10% H₂-85% N₂ atmosphere at 37°C for at least 7 days. This allowed detection and quantification of *P. gingivalis* colonies. The presence of *P. gingivalis* was confirmed by the characteristic black colonies and foul odor on the blood agar plates. Duplicate plates were incubated in the normal atmosphere at 37°C, and any animals with suspected contamination by bacteria other than *P. gingivalis* in their fluid samples were excluded from the study.

Cytokine analysis. For cytokine analysis, chamber fluids were processed for an enzyme-linked immunosorbent assay to detect mouse TNF, interleukin-1 β (IL-1 β), and gamma interferon (IFN- γ) as instructed by the manufacturer (Endogen, Rockford, IL). All mediator determinations were carried out in duplicate. The average of the two determinations was used for calculation of the amount of each mediator.

PGE₂ measurements. A direct competitive enzyme-linked immunosorbent assay kit (Neogen, Lansing, MI) was used to measure chamber fluid PGE₂ contents. The extent of color development was inversely proportional to the amount of analyte in the sample or standard. The bound enzyme conjugate was detected by addition of the K-Blue substrate, which generated optimal color at 650 nm. All determinations were carried out in duplicate.

Data analysis. Data analysis was performed using a statistical software package (SigmaStat, Jandel Scientific, CA). One-way analysis of variance was used to test the significance of the differences between the treated groups. When significance was established, the intergroup differences were tested for significance using Student's *t* test with the Bonferroni correction for multiple testing. Kaplan-Meier survival analyses with the log rank test were used in chamber exfoliation analyses of different groups. Z tests with the Yates correction were performed to examine the effects of TH on chamber exfoliation in DCM. The level of significance was a *P* value of <0.05. All the results are presented below as means \pm standard errors of the means.

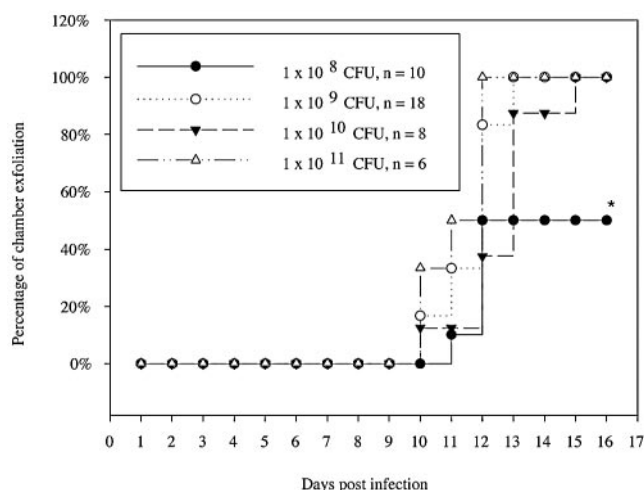


FIG. 1. Percentages of chamber exfoliation after infection with various inocula of *P. gingivalis* 381. The asterisk indicates that there was a significant difference between animals infected with 1×10^8 CFU and animals infected with other three larger bacterial challenge inocula (as determined by Kaplan-Meier survival analysis and log rank tests).

RESULTS

Chamber exfoliation. As shown in Fig. 1, intrachamber inoculation with 1×10^9 CFU of *P. gingivalis* 381 in 100 μ l BHI broth resulted in local suppurative lesions that ultimately proceeded to skin rupture accompanied by exfoliation of the infected chambers between day 10 and day 13 in 100% of the animals ($n = 18$), and the median day of chamber exfoliation (CE₅₀) was day 12. Our data also indicated that inoculation with 1×10^{11} , 1×10^{10} , 1×10^9 , and 1×10^8 CFU of *P. gingivalis* resulted in 33%, 12.5%, 16.7%, and 0% chamber exfoliation by day 10 after bacterial infection, respectively.

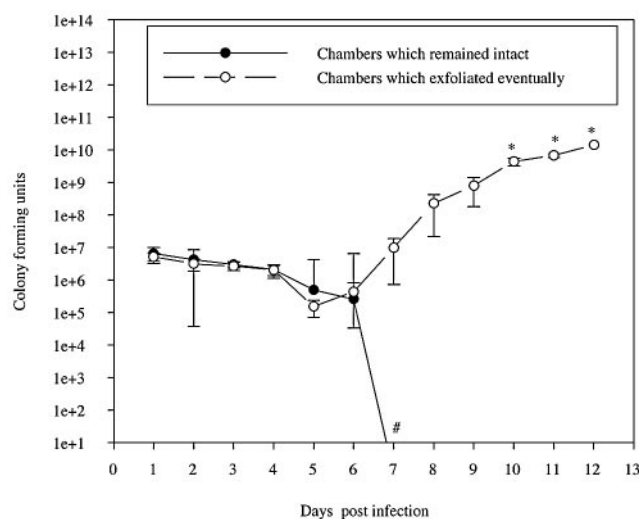


FIG. 2. Numbers of recoverable bacteria in chamber fluids following infectious challenge with 1×10^8 CFU *P. gingivalis*. The asterisks indicate that there were significantly more bacteria in day 10, 11, and 12 chamber fluids as determined by one-way analysis of variance and Bonferroni *t* tests ($n = 5$). The number sign indicates that we failed to retrieve bacteria from chamber fluids after day 7.

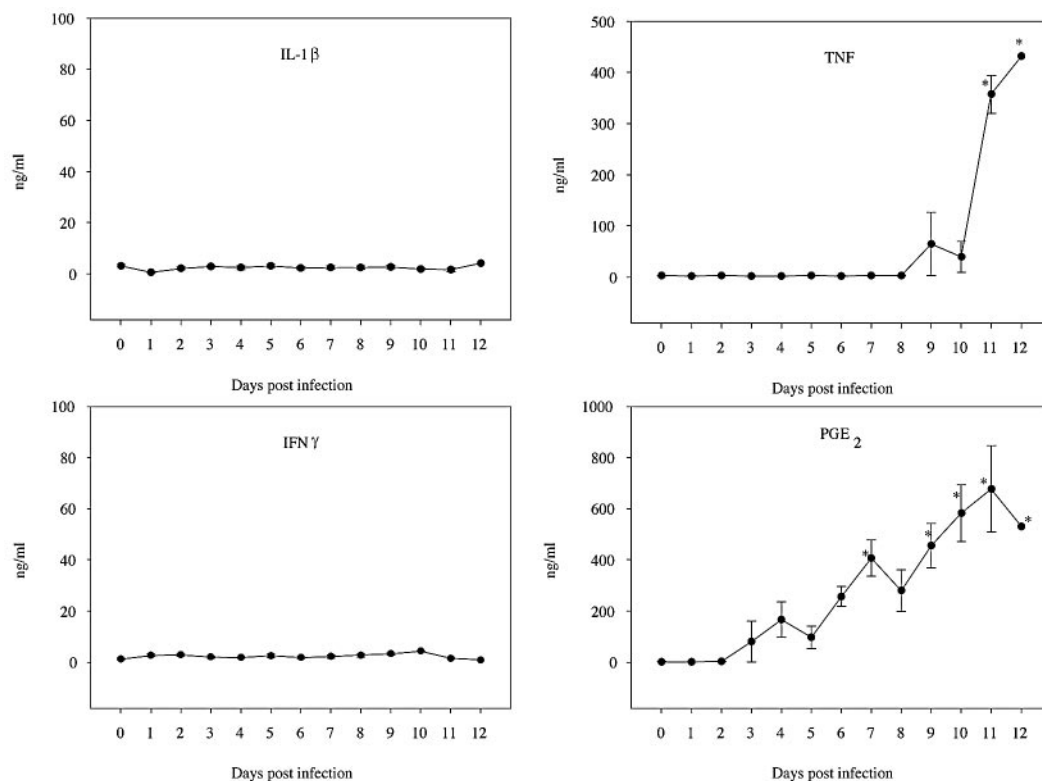


FIG. 3. Detection of various proinflammatory host mediators in chamber fluids following infectious challenge with 1×10^9 CFU *P. gingivalis*. The asterisks indicate that there were significant differences in day 11 and 12 TNF titers and in day 7, 9, 10, 11, and 12 PGE₂ titers in chamber fluids compared to day 0 fluids (as determined by one-way analysis of variance and Bonferroni *t* tests; $n = 6$ for each group).

However, increasing the number of challenge bacterium to 1×10^{10} or 1×10^{11} CFU did not change the ultimate pathological outcome, as for 100% of the animals the chambers were exfoliated on almost the same day that they were exfoliated by animals that were challenged with 1×10^9 CFU of *P. gingivalis* 381, as determined by Kaplan-Meier survival analysis with log rank tests (Fig. 1). Reducing the number of challenge bacteria to 1×10^8 CFU resulted in a 50% reduction in the number of animals with exfoliated chambers, but the CE₅₀ was not different from that of animals challenged with 1×10^9 CFU bacteria (Fig. 1).

Numbers of recoverable bacteria in infected chambers. *P. gingivalis* was recovered daily from infected chambers to monitor the change in number until chamber exfoliation. Bacteria were present in all the infected chambers, but the number gradually declined until day 6 following challenge with 1×10^8 CFU of *P. gingivalis* (Fig. 2). The decreases in the number of recoverable bacteria were statistically significant in day 5 fluids compared to day 1 fluids. There was a clear distinction between chambers which remained intact and chambers which were exfoliated eventually in terms of the number of recoverable bacteria after day 6 postinfection. For the exfoliated chambers, *P. gingivalis* multiplied rapidly and the concentration reached a plateau of 1.43×10^{10} CFU/ml before chamber exfoliation (Fig. 2). In contrast, for the intact chambers bacteria disappeared from the chamber fluids after day 6 postinfection, and these chambers remained bacterium free until the end of the experiment (Fig. 2). For chambers challenged with 1×10^9 , 1

$\times 10^{10}$, or 1×10^{11} CFU of *P. gingivalis* 381 there was 100% chamber exfoliation, and the data also generated curves for daily numbers of recoverable bacteria similar to the curves obtained for exfoliated chambers challenged with 1×10^8 CFU (data not shown).

TNF, IL-1β, IFN, and PGE₂ measurements. Three proinflammatory cytokines, TNF, IL-1β, and IFN-γ, were examined in this study. We did not detect any significant changes in IL-1β and IFN-γ levels in chamber fluids on a daily basis (Fig. 3). On the other hand, elevated titers of TNF were detected in chamber fluids after day 9, and the titers peaked significantly in day 12 fluids, the day just prior to chamber exfoliation (Fig. 3). For PGE₂, another proinflammatory mediator molecule, there was a steady increase in chamber fluids following *P. gingivalis* 381 infectious challenge, which escalated throughout the pathological course. There were significant increases after day 6 compared to day 1 fluids (Fig. 3).

TH and IN effects on chamber exfoliation. Since the proinflammatory mediator profiles indicated that TNF and PGE₂ could be involved in the infection process as pathology progressed, especially at the stage of tissue destruction resulting in chamber exfoliation, it was logical to check the effects of TNF and PGE₂ on the pathological outcome in this infection model. We blocked the production of either factor during bacterial infection by intraperitoneal injection of TH (a TNF inhibitor) or IN (a prostaglandin inhibitor). There was 100% chamber exfoliation by day 15 after bacterial challenge in the group that received daily IN injections, and the results were not different

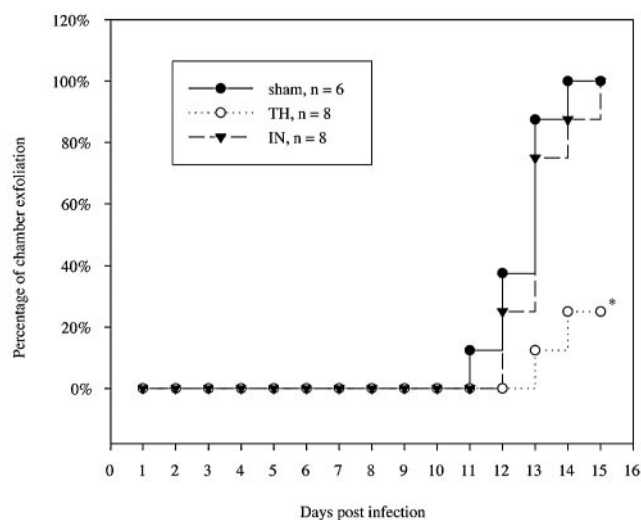


FIG. 4. Chamber exfoliation after *P. gingivalis* (1×10^9 CFU) infection in mice treated with TH (100 μ g/day) or IN (500 g/day). The asterisk indicates that in 25% of TH-treated mice the chambers were retained following *P. gingivalis* 381 challenge, which was significantly different from the results for the sham control group (as determined by Kaplan-Meier survival analysis and log rank tests).

from the results obtained for the sham group (Fig. 4). On the other hand, daily injection of TH reduced chamber exfoliation 75% ($n = 8$). Most animals that received TH injections still retained their chambers without additional pathology, and recovery of bacteria from these intact chambers was negative at the end of the experiment (data not shown).

Exogenous TNF and PGE₂ effects on chamber exfoliation in noninfected mice. To determine the roles of TNF or PGE₂ in the development of chamber exfoliation, exogenous TNF or PGE₂ was introduced into the experimental model without bacterial infection. The results showed that in the absence of *P. gingivalis* neither treatment initiated or caused tissue destruction based on an evaluation of the time of chamber exfoliation, and no chamber in either group was exfoliated by day 30 (data not shown).

Exogenous TNF and PGE₂ effects on uninfected chambers in locally infected mice in DCM. To further understand the contribution of TNF to the local tissue destruction caused by *P. gingivalis* 381 infection, we developed a novel animal model with two chambers inserted subcutaneously on the back of each mouse. Using this design, we challenged one chamber with *P. gingivalis* 381 (the infected chamber), and 9 days later the other chamber (the uninfected chamber) was challenged with either exogenous TNF or PGE₂ or with PBS as a control. We found that 67% of the exogenous TNF-treated uninfected chambers in infected animals were exfoliated by day 16 (Fig. 5B, left panel), even though no bacteria were present in these uninfected chambers (as confirmed by bacterial cultivation), indicating that chamber exfoliation was not a direct outcome of bacterial activity. Conversely, uninfected chambers in infected animals that were sham treated or were treated with exogenous PGE₂ did not exfoliate (Fig. 5A and C, left panel) and showed no signs of infection and inflammation. We observed no recoverable bacteria or inflammatory cell infiltration by microscopic examination of chamber fluids.

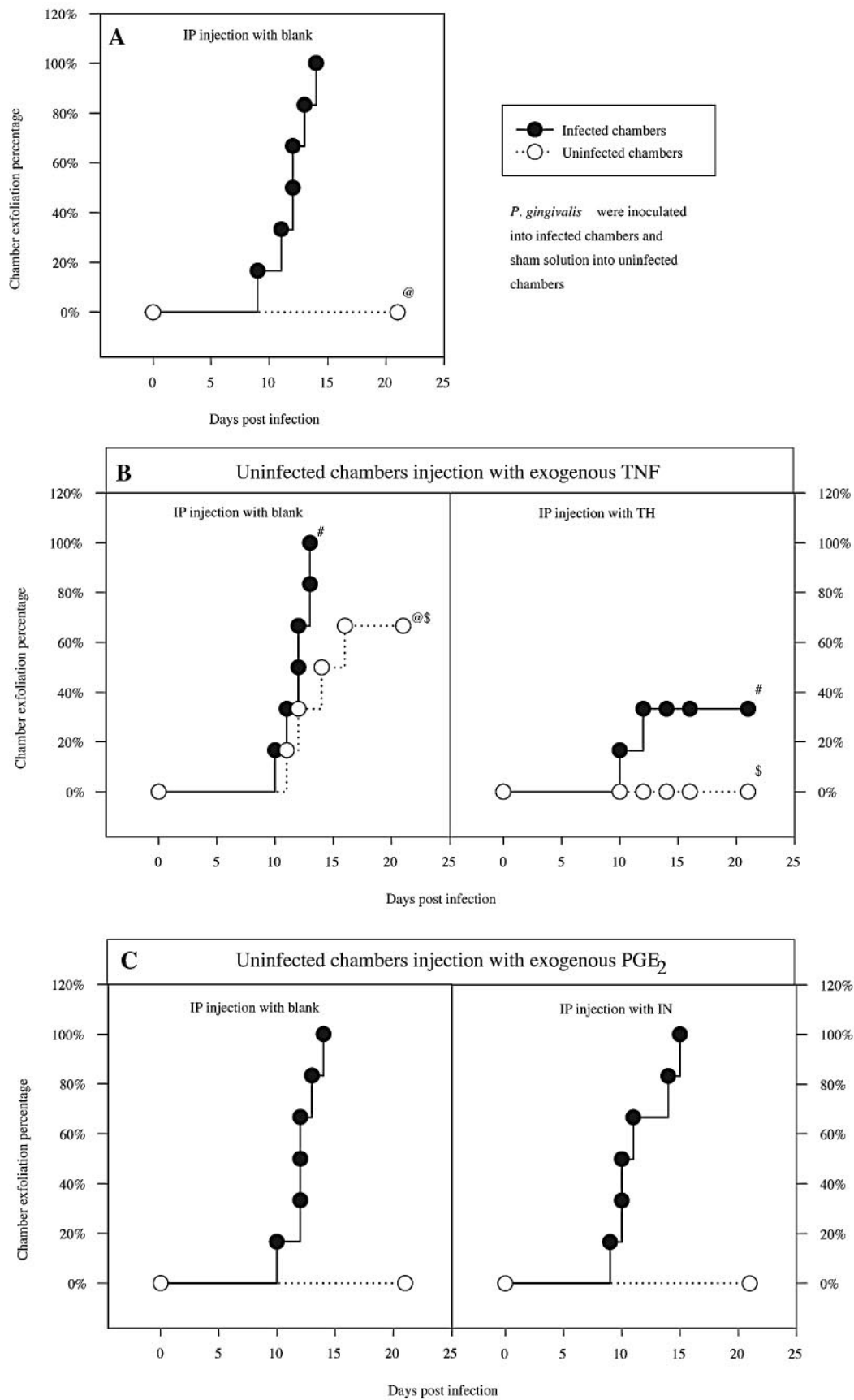
TH and IN effects on chamber exfoliation in DCM. A 67% reduction in chamber exfoliation was seen in the group that received the TH treatment (Fig. 5B, right panel). Noticeably, TNF-inducing chamber exfoliation was totally inhibited by TH (Fig. 5B, right panel). There were no signs of bacterial dissemination to nonchallenged chambers, as no *P. gingivalis* was recovered from day 11 nonchallenged chambers. IN treatment did not alter the outcome of chamber exfoliation, as infected chambers and uninfected chambers behaved just like the corresponding controls (Fig. 5C, right panel).

TH effects on uninfected chamber exfoliation in DCM. Uninfected chambers in the DCM were inoculated with various bacterium-associated molecules to further validate the role that TNF plays in the chamber exfoliation process. Exogenous TNF resulted in about 66.67% ($n = 6$) aseptic chamber exfoliation in the presence of local infection in the same animals. However, under the influence of TH treatment, exogenous TNF did not cause any chamber exfoliation (Fig. 6). Injection of *E. coli* LPS, a potent TNF inducer, into the animals resulted in 50% chamber exfoliation, and TH abrogated the effects of LPS and reduced the exfoliation percentage to 12.5%. A representative bacterial peptide, fMLP, had an effect on chamber exfoliation similar to that of exogenous TNF, but TH prevented only 50% of the chambers from exfoliating. Injection of live *P. gingivalis* into both chambers in the same animal resulted in 100% chamber exfoliation with or without the influence of TH. It was interesting that TH treatment did result in some differences in the development of chamber exfoliation, not in the percentage of chamber exfoliation but in the timing of chamber exfoliation. In the absence of TH, all chambers exfoliated by day 15 with a CE₅₀ of 12 days, while with the TH treatment, not all chambers exfoliated until as late as day 25 and the CE₅₀ was 19 days (Fig. 6). Heat-killed *P. gingivalis* was incapable of inducing chamber exfoliation, even when it was injected at the same level as live *P. gingivalis* (Fig. 6).

DISCUSSION

The subcutaneous chamber model described here is a model for the host tissue destruction that occurs in periodontitis. We hypothesized that the host reactions to periodontopathogen infection in a subcutaneous chamber are similar to those around the periodontium. *P. gingivalis* 381 infection in this animal model caused local inflammation with leukocyte infiltration and exudate accumulation that ultimately led to a discrete pathological outcome consisting of skin rupture and chamber exfoliation. Infected chambers were all exfoliated between day 10 and day 15 (Fig. 1). Chamber exfoliation thus was used as a convenient indicator of tissue destruction in this study to elucidate the pathological mechanism of host responses to *P. gingivalis* infection resulting in tissue damage.

After examination of local inflammatory mediators in the chamber fluids, PGE₂ and TNF appeared to be connected to the pathogenesis of *P. gingivalis* 381 infection, as the levels of PGE₂ in chamber fluids steadily increased with infection and the levels of TNF peaked at the time of chamber exfoliation. Systemically inhibiting the effects of PGE₂ with a nonsteroid anti-inflammatory drug, IN, did not influence the pathology of *P. gingivalis* infection, and all chambers exfoliated within the same time range as they did in the control. On the other hand,



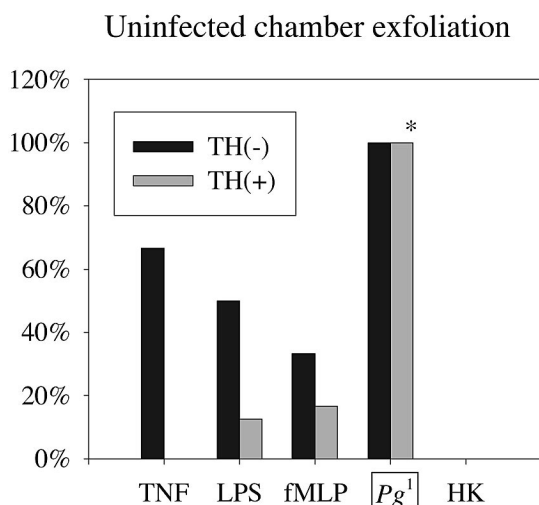


FIG. 6. Effects of TH on chamber exfoliation for uninfected chambers due to various treatments using the dual-chamber model. TH treatment resulted in a reduced percentage of chamber exfoliation for exogenous intrachamber injection of TNF, LPS, or fMLP, although the differences were not statistically significant as determined by Z tests with the Yates correction ($n = 6$). *P. gingivalis* (1×10^9 CFU) was also inoculated into both chambers of the same mouse (*Pg*¹). The asterisk indicates that TH treatment did not change the chamber exfoliation percentage for live *P. gingivalis* infectious challenge but increased the CE₅₀ from 12 to 19 days. HK, heat-killed *P. gingivalis*.

when TNF was blocked by TH, only 25% of the infected chambers were exfoliated (Fig. 4), suggesting the TNF plays a role in the development of chamber exfoliation.

To further understand the effects of host TNF on the progress of chamber exfoliation, the animal DCM was developed. We were inspired to modify the existing animal model by the observation that exfoliation of some noninfected chambers was induced by the transfer of fluids from infected chambers to noninfected chambers in the same animals (unpublished observation). The DCM that allowed us to monitor the effects of bacterial infection on hosts without the interference of bacterial products provided a unique tool with which to explore the role of host reactivity in the immune responses to *P. gingivalis* infection in this study. With this design, exogenous TNF alone was sufficient to induce leukocyte infiltration, chamber swelling, and chamber exfoliation if *P. gingivalis* coexisted in the same animal but not necessarily at the same site of TNF administration. The tissue damage effect was not restricted to TNF, inasmuch as *E. coli* LPS or fMLP also induced similar phenomena, but it was not totally inhibited by TH administration, indicating that factors other than TNF may also be involved in LPS- or fMLP-induced tissue destruction.

TNF has been shown to be produced in response to periodontopathogen infection by oral keratinocytes, gingival fibroblasts, monocytes, macrophages, and endothelial cells in vitro studies (12, 24). The TNF level was elevated during the acute phase of infection or the initial phase of infection (22). In this study, we found that the TNF level was also elevated during the time that infection-inducing tissue destruction occurred. TNF has a plethora of tissue-injuring properties, such as phagocyte recruitment (7) and subsequent release and activation of host metalloproteinases (13), fibrinolysis by activation of tissue plasminogen activator (27), and cell apoptosis (20, 23). TNF is produced in response to bacterial challenge and bacterial products, especially lipopolysaccharide (1). The initial response to TNF is to stimulate the recruitment of inflammatory cells through induced expression of chemotactic mediators, such as chemokines, and by stimulating the expression or activation of cell adhesion molecules on both leukocytes and endothelial cells. The local cellular effects of TNF include the capacity to induce neutrophils to bind to vascular endothelium, the capacity to activate phagocytosis, the capacity to elaborate superoxide bursts, and the capacity to promote degranulation (15). It is currently believed that matrix metalloproteinases are of major importance in tissue destruction. The effect of TNF on many of these enzymes is well known in vitro, since TNF up-regulates their synthesis. TNF may activate tissue fibrinolysis, as assessed by increased levels of tissue plasminogen activator, which in turn may activate tissue levels of thrombin. In animal models of arthritis, such as induced arthritis and TNF transgenic arthritis, anti-TNF therapy diminishes or prevents joint destruction (29). The effect of TNF inhibition therapy was shown to reduce the previously elevated serum levels of TNF (16). This suggests that TNF blockade may reduce matrix metalloproteinase synthesis and thus tissue damage. Free radicals are also induced upon activation of the oxidative burst by TNF (21). Superoxide anion generated by the action of a membrane enzyme, NADPH oxidase, has antimicrobial activity but can also be toxic to nearby cells. Blocking the effect of TNF by TH-alleviated tissue destruction in our study indicated that at least some of the properties mentioned above were involved in the chamber exfoliation process.

The tissue damage effects of TNF have now been implicated in a spectrum of pathogenic inflammatory states, including cachexia, autoimmune disease, rejection of organ transplants, and infections (25). Our results indicate that TNF is involved in the development of local lesions. The presence of TNF at the sites of infection at the time of chamber exfoliation reflects the important role of this cytokine in the control of the infection, probably as part of the innate immune response to control periodontopathogen multiplication, spread, or dissemination.

FIG. 5. Chamber exfoliation in dual-chamber model. For each mouse two chambers were implanted; one was infected with *P. gingivalis*, and the other was not infected. Uninfected chambers received a sham solution (A). The uninfected chambers received exogenous TNF (B). The uninfected chambers received exogenous PGE₂ (C). A @ symbol indicates that exogenous TNF resulted in 66.67% uninfected chamber exfoliation, which was significantly different from the results for the sham control-treated uninfected chambers (0%). A number sign indicates that intraperitoneal injection of TH significantly reduced chamber exfoliation to 33.33% compared to the value for the group that received intraperitoneal blank injection in the presence of TNF (100%). A dollar sign indicates that treatment with exogenous TNF resulted in total chamber retention, which was significantly different from the results for the group that received intraperitoneal blank injection in the presence of TNF (66.67% chamber exfoliation). IP, intraperitoneal.

The microbicidal mechanisms of phagocytes, with production of TNF and other cytokines, seem to be primarily responsible for the necrotic lesions of infection.

In summary, we concluded that TNF plays an important and complex local role at the infected sites in response to infection with *P. gingivalis*. It appears to be an important modulator of tissue inflammation. TNF activity in the animals in the course of infection leads to exaggerated inflammation and immune activation that are out of proportion to the increase in bacterial tissue burden, with resultant chamber exfoliation and tissue injury. More importantly, our data clearly suggest that there is a systemic effect of *P. gingivalis* local infection. The presence of *P. gingivalis* 381 modified the host tissue reactions to TNF administration. Without *P. gingivalis* 381 infection, TNF alone cannot cause tissue destruction in our experiment model. Only in the presence of *P. gingivalis* 381 infection can TNF mount the necessary tissue inflammatory reactions which lead to chamber exfoliation. Our results suggest that host reactions to periodontopathogens, like *P. gingivalis*, may be more prominent in periodontitis patients than in healthy individuals. With multiple infection foci in one animal (more than one tooth with periodontitis), patients may respond less favorably to immunotherapy targeting of TNF (Fig. 6). Our preliminary data obtained with dual-chamber experiments indicated that fewer bacteria were needed to induce exfoliation of one chamber if the other chamber in the same animal was coinfecting (data not shown). It is clear from our data that the local production of TNF can influence the pathogenesis of a particular inflammatory state or disease and that TNF plays a major role in tissue destruction in *P. gingivalis* infection.

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